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THE VELIGER

Spawning, Egg Mass Formation, and Larval Development of the Trochid Gastropod *Margarites helicinus* (Fabricius)

by

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Abstract. Spawning, egg mass production, and larval development through metamorphosis and hatching of the trochid gastropod, *Margarites helicinus* were observed. Eggs were released in a mucous string which was formed into an egg mass by the foot of the spawning female and attached to a substrate via mucous threads. One or more male snails were always in attendance during egg release and egg mass formation, but release of sperm was not seen. Larvae bypassed planktonic stages and crawled from egg masses as metamorphosed snails after 12 days. Egg masses produced by *M. helicinus* from the San Juan Islands (Washington, U.S.A.) were larger and contained more eggs than those produced by populations from the United Kingdom and Greenland. The development of larvae from all populations, however, is identical.

INTRODUCTION

The reproduction of *Margarites helicinus* (Fabricius) from the Pacific coast of North America has not been studied in detail. This paper presents a description of spawning, egg mass formation, and development for *M. helicinus* collected in the San Juan Islands, Washington, U.S.A., in March and April of 1985. The egg masses and development of *M. helicinus* from the San Juan Islands are also compared with descriptions of snails from the United Kingdom (JEFFREYS, 1865; FRETTER, 1955) and Greenland (THORSON, 1935).

MATERIALS AND METHODS

The individuals of *Margarites helicinus* used in this study spontaneously spawned egg masses in running seawater in the laboratory and attached them to the broad-bladed brown alga *Agarum fimbriatum* (Harvey, 1862). Nine of these egg masses were selected for observation. The number of eggs per mass was counted and the size of each mass was measured. Portions of blades bearing the newly laid egg masses were cut out and isolated in beakers which were then put in running seawater (7-9°C). A pipette was used to take samples from the nine egg masses periodically so that the timing and description of the developmental stages could be recorded.

Several more egg masses not used to determine patterns of development were left untouched so that changes in physical appearance could be observed. These egg masses were then compared to masses collected in the field.

RESULTS

Spontaneous spawning was observed in the laboratory from late March through April 1985 in water that was 7-9°C. *Margarites helicinus* egg masses were also seen on *Agarum* blades in the field during the same time period.

Prior to spawning two to four snails moved close together. A female then began to release eggs in a mucous string that was 2 or 3 eggs wide. Ejaculation of sperm was not observed during egg release or egg mass formation. Eggs were formed into a mass by the female's foot. The leading edge of the foot was repeatedly extended up into the mantle cavity and then moved down toward the substrate, molding recently released eggs onto the side of the existing mass (Figure 1A). The method of attaching the egg mass to the substrate was not observed, but finished egg masses were anchored at both ends and occasionally at additional points by mucous threads (Figure 1B). Egg mass formation lasted 0.5-1.5 h. After the egg masses were completed, the snails that did not release eggs, but were

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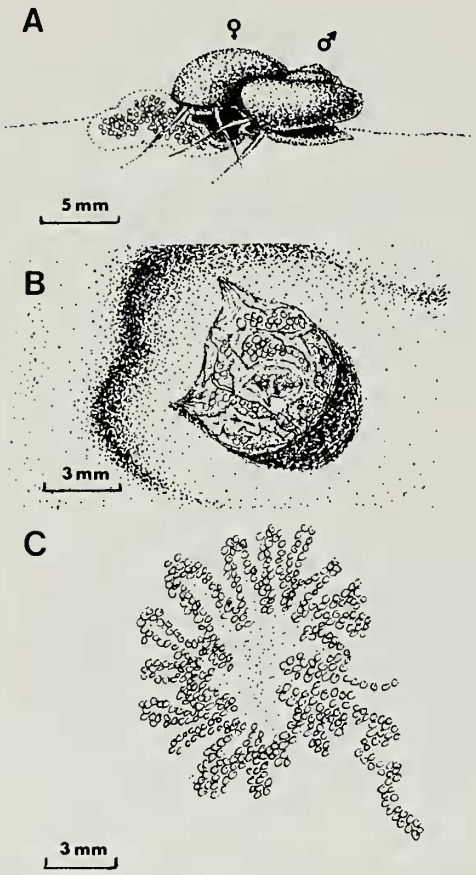

Figure 1

Egg mass production by *Margarites helicinus*. A. Male and female snails in close proximity during egg release and egg mass formation. B. A completed egg mass with mucous thread anchors attached to a depression on an *Agarum* blade. C. An egg mass compressed between a microscope slide and a cover slip.

associated with a spawning female, were sexed and found to be males.

The nine egg masses contained 95–377 eggs per mass ($\bar{x}$ = 239.2; SD = 101.3), ranged from 5–8 mm in diameter ($\bar{x}$ = 6.53 mm; SD = 1.08), and were spherical to oblong in shape. Eggs within the masses were arranged in a distinctive pattern, readily seen when a mass was pressed between a microscope slide and cover slip (Figure 1C).

Egg masses not used to follow development remained in the seawater table and soon collected particulate matter on the outer layer of mucus, obscuring many of the eggs from view. Egg masses from the field also had particulate matter covering much of their surfaces.

Eggs were 180 μm in diameter and orange to pinkish red in color. Each egg was surrounded by a 6–35 μm wide space bounded by a fertilization membrane and a transparent gelatinous coat 65–130 μm thick (Figure 2A).

Sperm were visible throughout the egg masses, between gelatinous coats of eggs, and at egg and fertilization membranes just after the egg masses were formed. Nearly 100% of the eggs in the sample were fertilized.

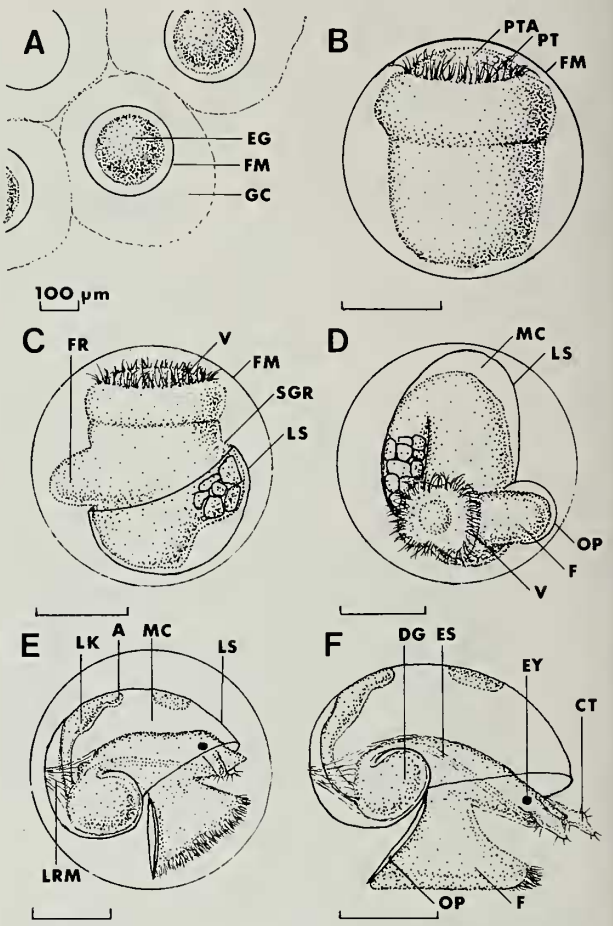

Figure 2

Developmental stages of *Margarites helicinus*. All scale bars represent 100 μm. A. Fertilized egg in an egg mass: EG, egg; FM, fertilization membrane; GC, gelatinous coat. B. Trochophore: FM, fertilization membrane; PTA, pretrochal area; PT, prototroch. C. Early veliger: FM, fertilization membrane; FR, foot rudiment; LS, larval shell; SGR, shell gland region; V, velum. D. Veliger midway through torsion: F, foot; LS, larval shell; MC, mantle cavity; OP, operculum; V, velum. E. Metamorphosed snail: A, anus; LK, larval kidney; LRM, larval retractor muscle; LS, larval shell; MC, mantle cavity. F. Hatched snail: CT, cephalic tentacle; DG, digestive gland; ES, esophagus; EY, eye; F, foot; OP, operculum.

Development proceeded in a spiral cleavage pattern. The first two cleavages were meridional, equal, and holoblastic. The third cleavage was equatorial and unequal. After two days the developing larvae became trochophores (Figure 2B). For a timetable of development see Table 1.

During the trochophore stage the foot rudiment and shell gland formed and the larval shell became visible. The prototroch then began to enlarge, forming a velum, and the larva became a veliger (Figure 2C).

During the veliger stage the foot, shell, and velum continued to develop and an operculum was formed. The velum enlarged into a bilobate structure. Within the region of the digestive gland several large (possibly yolky) cells

persisted. Veligers also underwent torsion, which took 0.5–1.5 days to complete (Figure 2D).

Prior to metamorphosis the bottom of the foot was ciliated, cephalic tentacles protruded through the velum, and eyes were visible at the base of the tentacles. During metamorphosis the velum was sloughed off and the snails were able to withdraw into their shells (Figure 2E). Two days after metamorphosis the snails crawled out of the egg masses (Figure 2F).

The entire developmental process took 12 days.

DISCUSSION

Margarites helicinus from the San Juan Islands spawns in the early spring, as was evidenced by spontaneous spawning in the laboratory in March and April and by the presence of *M. helicinus* egg masses on *Agarum* in the field during the same time period. This is 2–3 months earlier than spawning by *M. helicinus* populations in Greenland fjords, which spawn in the late summer months (THORSON, 1935).

Substrate selection for egg attachment is similar for populations in the San Juan Islands, Greenland, and the United Kingdom. Egg masses are attached to blades of algae: *Agarum* in the San Juan Islands, *Fucus* sp. and *Laminaria* sp. in Greenland (THORSON, 1935), and assorted algae in the United Kingdom (JEFFREYS, 1865; FRETTER, 1955). Snails from the United Kingdom also attach egg masses to the undersurfaces of stones (JEFFREYS, 1865; FRETTER, 1955). While the locations of egg mass deposition are quite similar, egg mass characteristics differ among the three populations.

The San Juan Islands population produces egg masses that are larger ($\bar{x}$ = 6.53 mm) and contain more eggs ($\bar{x}$ = 239) than masses produced by the other populations. The Greenland and United Kingdom populations produce masses that are 2–3 mm in diameter and hold 100–200 eggs (THORSON, 1935; FRETTER, 1955). The physical appearance of eggs also differs.

Eggs from all populations are the same size (180 μm), but eggs produced by snails from the San Juan Islands are orange to pinkish red in color, while Greenland snails produce yellowish-white eggs (THORSON, 1935). JEFFREYS (1865) reported that *Margarites helicinus* produces eggs in yellow membranous capsules, certainly an error, but did not mention egg color in his description.

Eggs within newly formed masses were surrounded by sperm. Sperm was most likely supplied by the attending male(s) as eggs were released and molded onto the egg mass. Although ejaculation of sperm was not seen, sperm were probably released very close to the egg-bearing mucous string. This would result in fertilization and explain the presence of sperm throughout the egg mass as well as aggregations of males around spawning females.

It is doubtful that sperm were transferred via spermatophore and that fertilization was accomplished within the female system before egg release for two reasons. First, one or more males were present during each spawn and left only after the egg mass was completed. Second, spermatophore transfer is known to occur in only one species of archeogastropod (FRETTER & GRAHAM, 1962).

Table 1

Timetable of the development of *Margarites helicinus*. Times are mean values for nine egg masses at 7–9°C.

Time	Stage
0.0 h	egg mass formation and fertilization
5.6 h	1st cleavage
12.5 h	2nd cleavage
18.1 h	3rd cleavage
1.3 days	gastrula
2.2 days	trochophore
2.7 days	shell formation
3.3 days	veliger
4.4–5.0 days	torsion
10.5 days	metamorphosis
12.1 days	hatching

Development in *Margarites helicinus* from the San Juan Islands is similar to that reported by THORSON (1935) and FRETTER (1955). The embryo passes the trochophore and veliger stages in the egg mass and emerges as a metamorphosed snail with a shell having 1¼ whorls and a diameter of 250 μm.

In conclusion, *Margarites helicinus* from the San Juan Islands, Washington, spawns earlier in the year and forms egg masses that are larger and contain more eggs per mass than their counterparts from Greenland and the United Kingdom. Larvae from all three locations bypass planktonic stages and emerge from egg masses as metamorphosed snails.

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